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Importance of Neonatal FcR in Regulating the Serum Half-Life of Therapeutic Proteins Containing the Fc Domain of Human IgG1: A Comparative Study of the Affinity of Monoclonal Antibodies and Fc-Fusion Proteins to Human Neonatal FcR

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The neonatal FcR (FcRn) binds to the Fc domain of IgG at acidic pH in the endosome and protects IgG from degradation, thereby contributing to the long serum half-life of IgG. To date, more than 20 mAb products and 5 Fc-fusion protein products have received marketing authorization approval in the United States, the European Union, or Japan. Many of these therapeutic proteins have the Fc domain of human IgG1; however, the serum half-lives differ in each protein. To elucidate the role of FcRn in the pharmacokinetics of Fc domain-containing therapeutic proteins, we evaluated the affinity of the clinically used human, humanized, chimeric, or mouse mAbs and Fc-fusion proteins to recombinant human FcRn by surface plasmon resonance analysis. The affinities of these therapeutic proteins to FcRn were found to be closely correlated with the serum half-lives reported from clinical studies, suggesting the important role of FcRn in regulating their serum half-lives. The relatively short serum half-life of Fc-fusion proteins was thought to arise from the low affinity to FcRn. The existence of some mAbs having high affinity to FcRn and a short serum half-life, however, suggested the involvement of other critical factor(s) in determining the serum half-life of such Abs. We further investigated the reason for the relatively low affinity of Fc-fusion proteins to FcRn and suggested the possibility that the receptor domain of Fc-fusion protein influences the structural environment of the FcRn binding region but not of the FcyRI binding region of the Fc domain. The Journal of Immunology, 2010, 184: 1968–1976.

In healthy humans, IgG1 exhibits a long serum half-life of ∼21 d (1). This prolonged half-life of IgG can be explained by the interaction with neonatal FcR (FcRn). FcRn is a heterodimer of the MHC class I-like H chain and the β2-microglobulin (β2m) L chain (2). Although this receptor was originally studied as a transporter of IgG from mother to fetus, subsequent studies have shown that this receptor also plays a critical role in regulating IgG homeostasis (3, 4). FcRn binds to the Fc domain of IgG at pH 6.0–6.5 but not, or weakly, at pH 7.0–7.5 (5). Therefore, FcRn protects IgG from degradation by binding to IgG in endosome and releases IgG into plasma (6). As indicated by previous studies in which amino acid substitutions in the Fc domain of IgG for modifying the affinity to FcRn can alter the serum half-life of the IgG, the affinity to FcRn is thought to play a critical role in determining the serum half-life of IgG (7–12).

Recently, therapeutic use of mAb products has become more important for various diseases, including cancer as well as autoimmune and infectious diseases (6, 13, 14). In addition to the mAbs, the Fc-fusion proteins (e.g., etanercept, alefacept, and abatacept) have been developed and have received considerable attention. These Fc-fusion proteins consist of an extracellular domain of membrane receptor linked to the Fc portion of human IgG1. They work like Abs by binding to ligands for the receptors. The receptor portions of etanercept and alefacept are, respectively, the extracellular ligand-binding portion of the human 75-kDa TNFR and the extracellular CD2-binding portion of the human leukocyte function Ag 3. Abatacept consists of the extracellular domain of human CTLA-4 linked to the modified Fc portion of human IgG1.

Most of the mAb products and Fc-fusion protein products have the Fc domain of human IgG1 (6, 14). Accumulating evidence regarding their clinical use has revealed that their serum half-lives are variable, ranging from 4 to 23 d, regardless of the presence of the Fc domain of human IgG1 (6). Although many factors such as m.w., posttranslational modifications including glycosylation, electrical properties, interactions with FcRs or target molecules, and features of the target molecules may influence their serum half-life, the reasons for the variability of half-life have not been elucidated. Among such factors, FcRn might play a critical role in regulating half-life; however, comparative studies between the affinities of these therapeutic proteins to FcRn and their half-lives in humans have not been reported. Therefore, although some Fc domain-containing therapeutic proteins exhibit shorter half-lives in humans, it remains unclear whether the shorter half-lives are due to the lower affinity to FcRn or other factors.
In this study, we examined the affinity of clinically used mAbs and Fc-fusion proteins to recombinant human FcRn by surface plasmon resonance (SPR) analysis. The analytes used were human Ab (adalimumab), humanized Abs (dactizumab, omalizumab, palivizumab, and trastuzumab), chimeric Abs (infliximab and rituximab), mouse Ab (muromab-CD3), and Fc-fusion proteins (etanercept, alefacept, and abatacept). We found that the affinities of the therapeutic proteins tested to FcRn were closely correlated with their serum half-lives, with a few exceptions. Because Fc-fusion proteins, which have relatively short half-lives (4–13 d), were shown to have lower affinity to FcRn than mAbs, we further investigated the reason for this difference by examining the affinity of the proteins to FcγRI or the affinity of papain-digested proteins to FcRn in SPR analyses. Our results suggested the possibility that the receptor portions of Fc-fusion proteins make a difference in the higher-order structure of the FcRn-binding region of Fc (i.e., CH2-CH3 interface) or interfere with binding between the Fc domain and FcRn by steric hindrance.

Materials and Methods

Therapeutic proteins and reagents

Abatacept (Bristol-Myers Squibb, Princeton, NJ), adalimumab (Abbott, Baar, Switzerland), alefacept (Biogen Idec, Cambridge, MA), dactizumab (Hoffmann-La Roche, Nutley, NJ), etanercept (Takeda Pharmaceutical, Osaka, Japan), infliximab (Tanabe Pharmaceutical, Osaka, Japan), intriniximab (Zenyaku Kogyo, Tokyo, Japan), and trastuzumab (Chugai Pharmaceutical, Tokyo, Japan) were purchased via reagent distributors. Recombinant human TNF-α was purchased from Wako (Osaka, Japan).

Purification of human FcRn

Stably transfected CHO cells expressing both the soluble portion of the hFcRn H chain (residues 1–267 of mature protein) and β2m were provided by P. J. Bjorkman (California Institute of Technology, Pasadena, CA). Expression and purification of hFcRn were performed according to the method previously reported by West and Bjorkman (15), with slight modifications. Briefly, the CHO cells expressing soluble hFcRn and β2m were cultured in α-MEM containing 5% dialyzed FBS, 100 mM methionine sulfoximine, and penicillin/streptomycin. Cell culture supernatant was acidified to pH 2.0 with a 25% aqueous solution of 1 M HCl 0.05% and then diluted with 0.05% Tween 20 to a concentration of 0.5 mg/ml. The harvested supernatant was then treated with 50 mM Bis-Tris (pH 5.8), hFcRn complexed with azide was then added to 0.05% Tween 20. The supernatant was then treated with 50 mM Bis-Tris/20 mM Tris (pH 5.8), and then eluted with pH gradient using 40 mM Bis-Tris/20 mM Tris (pH 8.1) and 40 mM NaCl (pH 8.1) was injected for 2 min. Fc-fusion proteins were then purified using an anion-exchange column with a pH gradient of 40 mM NaCl [pH 8.1] to 300 mM NaCl [pH 6.8] for 30 min, respectively. Western blotting of the purified proteins was performed to confirm that the isolation procedures resulted in high-purity samples. The purified recombinant hFcRn was diluted with 10 mM sodium phosphate/150 mM NaCl (pH 6.0) and injected at 25°C. The running buffer was allowed to flow at a rate of 20 μl/min. The injections of papain-digested proteins were performed using the KINJECT mode (volume, 40 μl). Kinetic analyses of FcγRI binding were performed according to the method of Ellsworth et al. (16) with some modifications. The running buffer, HBS-EP (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, and 0.005% Surfactant P20 [pH 7.4]) (Biacore), was allowed to flow at 20 μl/min. The injections of FcγRI were performed using the KINJECT mode (volume, 40 μl; dissociation time, 150 s). To regenerate the immobilized proteins, the reagent buffer (10 mM glycine-HCl [pH 1.8]) was injected for 15 s.

Papain digestion

The papain (Wako) was activated in the buffer (50 mM sodium phosphate/150 mM NaCl [pH 6.0], 1 mM cysteine, 4 mM EDTA, and 1 mg/ml papain) at 37°C for 15 min. Next, 1 mg/ml Ab or Fc-fusion protein was digested with 0.1 mg/ml activated papain in 50 mM sodium phosphate (pH 6.0), 150 mM NaCl, 0.1 mM cysteine, and 4 mM EDTA at 37°C for 24 h.

Results

Purification of soluble human FcRn

FcRn binds to the Fc domain at acidic pH and then releases it at neutral pH. Recombinant soluble hFcRn expressed from CHO cells was purified using a human IgG column by binding at pH 5.8 and releasing at pH 8.1. The fraction purified by the IgG column was electrophoresed at lane 10 of SDS-PAGE gel (Fig. 1B). This fraction was then purified using an amion-exchange column with a pH gradient elution. The elution diagram is shown in Fig. 1A. Three main peaks were observed. The proteins in these peaks were electrophoresed (Fig. 1B) and subjected to Western blot analysis using anti-hFcRn H chain peptide Ab (Fig. 1C). Several bands were observed at ~32 kDa in these fractions, and these bands were immunoreactive to anti-FcRn H chain peptide Ab. These results indicated that the purified FcRn had several isoforms, possibly because of the difference in posttranslational modification, including glycosylation or proteolysis. As shown in Fig. 1C, the signals of the higher m.w. bands of hFcRn tend to be weak. There is a possibility that the sugar chain at Asn125 of hFcRn interfered with the reactivity of the hFcRn to the anti-hFcRn H chain peptide Ab used. We analyzed the affinity of therapeutic mAbs and Fc fusion proteins to FcRn by SPR using the peak I, II, or III fractions eluted from the anion-exchange column. The Kd values were higher when peak I was used as a ligand in SPR analyses than when peaks II or III were used (data not shown). Because the m.w. of the proteins in peak I was smaller than that in peak II/III and the protein content of peak I varied depending on the lot of the cell culture supernatant, peak I seemed to consist of immature FcRn. The Kd values calculated from the experimental data using peaks I and III were comparable (data not shown). We, therefore, used the main peak (i.e., peak III) in the following experiments.
Purified FcRn was immobilized onto a CM5 biosensor chip at relatively low densities as described in Materials and Methods. Five or six concentrations of Fc domain-containing therapeutic proteins were then injected. Because injection at higher concentrations caused nonspecific binding to flow cells, we analyzed the affinity of therapeutic proteins using sensorgrams obtained at the concentrations at which nonspecific binding was not observed. For example, infliximab was injected at concentrations of 670, 335, 168, 84, and 42 nM, and we analyzed the affinity to FcRn with the bivalent analyte model (Fig. 2). The colored lines were observed sensorgrams, and the black lines were fitting lines generated by the BIAevaluation software. The $K_D$ value ($=k_d/k_a$) calculated from these sensorgrams was 727 nM. The affinities of adalimumab and etanercept to FcRn were 672 and 3612 nM, respectively (Fig. 2).

The affinities of the 11 kinds of Fc domain-containing proteins to FcRn were measured (Fig. 3). Adalimumab, daclizumab, infliximab, palivizumab, and rituximab were injected at concentrations of 42–670 nM. The concentrations of abatacept, alefacept, and etanercept used were 168–5360 nM, and those of muromonab-CD3, omalizumab, and trastuzumab were 84–1340 nM. Under this condition, the tested therapeutic proteins, except for muromonab-CD3, bound to FcRn. The $K_D$ values measured in our experiments and the serum half-lives reported in the literature are shown in Fig. 3A.

The $K_D$ values and the average values of the serum half-lives are plotted in Fig. 3B. The $K_D$ values were closely correlated to the half-lives (contribution ratio = 0.8675) when the results were analyzed after excluding the data for infliximab, rituximab, and trastuzumab (Fig. 3C). Concerning infliximab, rituximab, and trastuzumab, which have relatively short half-lives and comparable affinity to other long half-life Abs to FcRn, other critical factor(s) seemed to be involved in regulating their half-lives (see Discussion). Although it was impossible to plot the data for mouse mAb muromonab-CD3,
which exhibited no significant binding to human FcRn, the half-life of this Ab in humans is the shortest (0.75 d) among the therapeutic proteins examined in this study (21). These results also show the importance of the binding affinity to FcRn in determining the serum half-life. The correlation described above was also observed when other fractions of hFcRn described in Fig. 1 (peaks I and II) were used in SPR analyses (data not shown).

The affinity between FcγRI and Fc domain-containing proteins

Because the affinities of Fc fusion proteins (etanercept, alefacept, and abatacept) to FcRn were lower than those of mAbs, the FcRn-binding region (CH2-CH3 domain interface) of Fc-fusion proteins seems to be structurally different from that of mAbs. We also analyzed the affinity of these proteins to FcγRI to test whether the structural environment around the FcγRI-binding region (hinge proximal region of CH2) is different between Fc-fusion proteins and Abs. Because the regeneration procedure in the SPR assay inactivated FcγRI but not Fc domain-containing therapeutic proteins, therapeutic proteins were immobilized to CM5 biosensor chips, and FcγRI was used as an analyte. The sensorgrams of Fc-fusion proteins (abatacept, alefacept, and etanercept) and mAbs (adalimumab and infliximab) are shown in Fig. 4A. The data were analyzed with a 1:1 binding model. The $K_D$ values of the two Fc fusion proteins (alefacept and etanercept) and Abs (adalimumab and infliximab) were comparable (Fig. 4B). The $K_D$ values obtained in this study were similar to the data reported for IgG [reviewed by van de Winkel and Anderson (26)]. In contrast, abatacept had a lower affinity to FcγRI. In abatacept, a series of selected mutations those can alter the binding affinity to FcγRI were introduced to reduce Fc-mediated cytotoxic effects (Fig. 5) (28, 29). Therefore, the data in Fig. 4 show that the change in the affinity of Fc domain to FcγRI, which is caused by amino acid substitutions, was detected in our experiments. These results suggest that the region interacting with FcγRI (i.e., the hinge proximal region of CH2) was not structurally different between Fc fusion proteins, except for abatacept, and Abs examined.

The affinity between FcRn and Fc domains generated by papain treatment

In Fig. 5, the amino acids sequences of abatacept, alefacept, etanercept, adalimumab, infliximab, and omalizumab are aligned. The differences in the primary structure of the Fc regions were Glu376 and Met 378 of etanercept, which are attributed to the IgG1 allotype, and Ser 162, Ser165, and Ser 174 of abatacept, which are due...
to the engineering for decreasing affinity to FcγR and improving protein production (28). To test the possibility that this limited structural difference or posttranscriptional modifications such as glycosylation can give rise to the difference in binding affinity to FcRn, we digested the Fc-fusion proteins or mAbs with papain and analyzed the affinity of their Fc domains to FcRn. The electrophoretic pattern of etanercept and adalimumab digested with papain is shown in Fig. 6A. Both etanercept and adalimumab were digested sufficiently for 24 h at 37 °C under the conditions described in Materials and Methods, whereas digestion was not sufficient after incubating for 2 h. Therefore, the therapeutic proteins digested with papain for 24 h were used for the SPR analyses. The sensorgrams of etanercept (670 nM) and adalimumab (670 nM) were much different without incubation with papain, but they became almost identical after papain digestion (Fig. 6B). We measured the affinities to FcRn of five therapeutic proteins digested with papain for 24 h were used for the SPR analyses. The sensorgrams of etanercept (670 nM) and adalimumab (670 nM) were much different without incubation with papain, but they became almost identical after papain digestion (Fig. 6B). We measured the affinities to FcRn of five therapeutic proteins (etanercept, alefacept, adalimumab, infliximab, and omalizumab) digested with papain (Fig. 6C). Etanercept and alefacept are Fc-fusion proteins with low affinity to FcRn, and omalizumab is an Ab showing lower affinity to FcRn than other Abs. Because it was possible that the proteins were cleaved, in part, into smaller fragments than the Fc domain, the estimated $K_D$ values may have been larger than the actual values. However, it was very clear that the affinities of etanercept, alefacept, infliximab, and omalizumab were increased by papain treatment (Fig. 6C).

The affinity of Fc-fusion protein and Abs became comparable after papain digestion, showing that the differences in amino acid sequences or posttranslational modification of the Fc domain did not contribute to the difference in the binding affinity of these proteins to FcRn. It therefore seems likely that the receptor domain of the Fc-fusion protein makes a difference in the higher-order structure of the FcRn-binding region of Fc (i.e., CH2-CH3 interface) or interferes with the binding between Fc domain and FcRn by steric hindrance. Moreover, such a difference or interference seems to be involved in determining the affinity to FcRn for some kinds of Abs, because the $K_D$ values of infliximab and omalizumab were also increased significantly by papain treatment.

The affinity between FcRn and therapeutic proteins binding with target molecules

On the basis of the results suggesting the possibility that another region besides the Fc domain influences the affinity of Fc domain-containing proteins to FcRn, we assumed that binding with the target molecule would also change the affinity to FcRn. Because adalimumab, infliximab, and etanercept bind to the same target molecule, TNF-α, we analyzed the effects of binding with TNF-α on the affinity of these therapeutic proteins to FcRn. First, 0–2680 nM TNF-α was added to 335 nM infliximab and incubated for at least 1 h. The resulting mixture was then injected into the flow cell, and the affinities to FcRn were analyzed. By adding TNF-α, the shape of the sensorgram was drastically altered (Fig. 7A). The Abs (adalimumab and infliximab) can maximally bind to two TNF-α trimers, whereas etanercept binds to one TNF-α trimer. When the relative concentrations of TNF-α are low, three molecules of the Ab can bind to each TNF-α trimer, and cross-linked TNF/Ab complexes are formed (30). To evaluate the affinity...
between FcRn and TNF-α–binding proteins, excess TNF-α was added to adalimumab, infliximab, and etanercept (8-fold molar excess to 42–670 nM Abs and 4-fold to 168–2680 nM etanercept) to avoid forming nonuniform complexes. The sensorgrams were fitted by the bivalent analyte model (Fig. 7B). Although the fitted lines did not completely match the observed sensorgrams, the $K_d$ values of infliximab, adalimumab, and etanercept to FcRn were calculated to be 2057, 1321, and 4286 nM, respectively (Fig. 7C). The affinity of infliximab–TNF-α complex or adalimumab–TNF-α complex was lower than that of infliximab or adalimumab, respectively (Fig. 7C). These results suggest that at least for these anti–TNF-α Abs, binding with target molecules decreases the affinity to FcRn. They may also suggest that the anti–TNF-α Abs complexed with TNF-α will be degraded more rapidly than anti–TNF-α Abs free from TNF-α in vivo.

Discussion

To our knowledge, this is the first article to elucidate the affinities of clinically used Fc domain-containing therapeutic proteins to FcRn in a comparative study. Because the affinities of these therapeutic proteins to FcRn were found to be highly correlated with the serum half-lives in humans, with the exception of infliximab, rituximab, and trastuzumab, the importance of FcRn in regulating the serum half-life of Fc domain-containing therapeutic proteins was suggested. The key observation was that the Fc-fusion proteins showed lower affinity to FcRn than Abs. These data provided us with one of the answers to the question of why the Fc-fusion proteins containing the Fc domain of human IgG1 exhibit a shorter half-life than human IgG1.

In the current study, we used the bivalent analyte model of BIAevaluation software. Most studies analyzing Fc-FcRn interactions have used the bivalent analyte model (15, 31) or the heterogeneous ligand model (7, 15, 31). Although the sensorgrams in our experiments were able to be fitted by both models, they were better fitted by the bivalent analyte model. Considering that two molecules of hFcRn bind to each IgG, resulting in a 2:1 binding stoichiometry (15), the bivalent analyte model seems to be suitable. It has been reported that the dual bivalent analyte model better fits the data of the FcRn-Fc interaction (32), although there are cases in which the bivalent analyte model does not work well. In the article about the dual bivalent analyte model, it was speculated that high-affinity and low-affinity types of FcRn existed on the surface of the BIACore chip and that the low-affinity-type receptor was probably an experimental artifact (32). Possibly because the content of the low-affinity-type FcRn on the chip is comparatively low in our immobilizing condition, the sensorgrams in our experiments might have been well-fitted by the bivalent analyte model.

Among the therapeutic proteins tested in this study, the Fc fusion proteins showed relatively lower affinities to FcRn (Figs. 2, 3), although the affinities to FcγRI are comparable to those of Abs (Fig. 4). Although the Fc domain binds to FcRn via the CH2-CH3 domain interface (33), the primary structures of the Fc domains of tested therapeutic proteins were almost the same, and cleavage of the Fc domains from Fab or the receptor region gave similar $K_d$ values to FcRn (Fig. 6). These results suggest that the receptor regions of Fc-fusion protein alter the conformation of the FcRn-binding region (CH2-CH3 domain interface), not of the Fc domain interface (33), the primary structures of the Fc domains of tested therapeutic proteins were almost the same, and cleavage of the Fc domains from Fab or the receptor region gave similar $K_d$ values to FcRn (Fig. 6). These results suggest that the receptor regions of Fc-fusion protein alter the conformation of the FcRn-binding region (CH2-CH3 domain interface), not of the Fc domain interface. The influence of regions besides the Fc domain on FcRn-binding regions would also be the case for Abs, as shown in Fig. 7.

Our results presented in this study can provide valuable information regarding the molecular design of novel Fc domain-containing therapeutic proteins and demonstrate the usefulness of FcRn-binding analysis in the characterization of Fc domain-containing therapeutic proteins. In addition to the Fc fusion proteins used in this study, rilonacept, a Fc-fusion protein consisting of ligand-binding domains of the extracellular portions of the human IL-1 receptor component (IL-1RI) and IL-1 receptor accessory protein linked to the Fc portion of human IgG1, and romiplostim,
a Fc-peptide fusion protein consisting of human IgG1 Fc domain linked at the C terminus to a peptide containing two thrombopoietin receptor-binding domains, were approved recently (34, 35). The

FIGURE 6. Effects of papain digestion on the affinities of Fc domain-containing therapeutic proteins to FcRn. A, The nonreduced SDS-PAGE of etanercept and adalimumab digested with papain for 2 and 24 h. B, The comparison between the sensorgrams of etanercept and adalimumab with or without papain digestion. C, Comparison of the affinity to FcRn among etanercept, alefacept, adalimumab, infliximab, and omalizumab, which were digested or not digested with papain. The $K_D$ values were calculated from the sensorgrams at the range of concentrations described as follows. The concentrations of papain-digested etanercept, papain-digested alefacept, adalimumab, papain-digested adalimumab, infliximab, papain-digested infliximab, and papain-digested omalizumab were 42–670 nM; those of etanercept and alefacept were 168–5360 nM, and those of omalizumab were 42–1340 nM. Each bar shows the average $K_D$ value + SD, which was calculated from three independent experiments.

FIGURE 7. Effects of binding with the target molecules on the affinities of Fc domain-containing therapeutic proteins to FcRn. A, The sensorgrams of infliximab (335 nM) preincubated with TNF-α (0–2680 nM). B, The sensorgrams of infliximab (upper panel), adalimumab (middle panel), and etanercept (lower panel) preincubated with TNF-α (8-fold molar excess to 42–670 nM Abs and 4-fold to 168–2680 nM etanercept). The sensorgrams were fitted by the bivalent analyte model. C, The $K_D$ values calculated from the sensorgrams shown in B. The values of infliximab, adalimumab, and etanercept derived from the same series of experiments are also shown as controls.

**NS, no significant difference according to Student $t$ test.**
development of Fc-fusion proteins will receive further attention. Although the Fc domains are used with the intent of prolonging the half-lives of receptor proteins, the half-lives tend not to be fully prolonged to the level of IgG1. It remains unclear whether the receptor regions of Fc-fusion proteins alter the conformation of the CH2-CH3 domain interface or the regions cause steric hindrance on the binding site of FcRn; however, the molecular design of Fc-fusion proteins having a higher affinity to FcRn might be possible in either case.

Reflecting the increasing interest in the development of mAbs and related products, the newly revised guideline for such products was adopted by the European Medicines Agency in 2008 (www.emea.europa.eu/pdfs/human/bwp/15765307enfin.pdf). In the guidelines, it is mentioned that FcRn-binding activity should be provided, as appropriate, in product characterization. Because regions other than the Fc domain might affect the affinity of the protein to FcRn (Figs. 6, 7), the affinity to FcRn should be evaluated as an important quality attribute related to the pharmacokinetic profile, even if the protein has a native Fc domain of IgG1, especially in cases of Fc-fusion proteins. Meanwhile, because it was demonstrated that oxidation of two labile methionines, Met252 and Met428, in human IgG1 attenuates binding of the Ab to FcRn (36), alteration of the affinity to FcRn during the production process or storage will reflect structural changes of the protein, including Met oxidation, that will lead to shortening the serum half-life. In addition to IgG, albumin is also known to bind to FcRn in a pH-dependent manner and is protected from degradation (37, 38). The albumin-fusion proteins (e.g., albumin-IFN) or drugs having an albumin-binding moiety are being developed. FcRn-binding characteristics would also be important as a quality attribute of such products, which is related to the pharmacokinetic profile.

As mentioned above, the existence of several Abs having a short half-life and high affinity to FcRn suggested the involvement of other critical factor(s) in regulating the serum half-life of Abs such as trastuzumab, rituximab, or infliximab. Trastuzumab is a humanized Ab directed against human epidermal growth factor receptor 2 (HER2), which is expressed in some types of breast cancer cells. It has been reported that trastuzumab is taken up by HER2-expressing cells via HER2-mediated endocytosis (39, 40). Rituximab, a chimeric Ab directed against CD20, is also internalized in an Ag-mediated manner (41). Because the ligand-dependent internalization is followed by degradation of Abs, this property seems to be an important reason for the short half-life of trastuzumab and rituximab. It has been reported that, in general, the half-life of monoclonal IgG Abs increases depending on the degree of humanization in the order of murine < chimeric < humanized < human (6, 41, 42). Because infliximab and rituximab are chimeric Abs, the involvement of common factors influencing the half-life of chimeric Abs such as the presence of human anti-chimeric Ab would be another reason for the shorter half-life.

As shown in Fig. 7, the affinities of infliximab–TNF-α complex and adalimumab–TNF-α complex seemed to be lower than those of infliximab and adalimumab. If the affinity of therapeutic proteins/target molecules complexes to FcRn is lower than that of the free therapeutic proteins, the complexes will be degraded faster. Therefore, the half-lives of such therapeutic proteins seem to be shortened in the case that the target molecules are abundant in the bodies of patients. In contrast, if the affinity to FcRn of therapeutic proteins/target molecule complexes is higher than that of the free drugs, the complexes of drug and target molecules will have longer half-lives than free drugs. Because there are many factors affecting the elimation of Abs [reviewed by Tabrizi et al. (41)], further studies are necessary to elucidate the critical factors impacting the half-lives of Fc domain-containing proteins, in addition to the affinity to FcRn. Binding characteristics of the Fc domain-containing proteins or their complex with target molecules to FcγRs would be one of the important issues to be examined in regard to the impact on their elimination.

In conclusion, we showed the importance of the affinity to FcRn in determining the serum half-life of Fc domain-containing therapeutic proteins. Further investigation regarding the molecular structures that regulate the affinity of the engineered protein to FcRn will accelerate the development of therapeutic proteins with a desired half-life.

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Disclosures
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